

### **COP Format for Annual Progress Report**

- A. Grant Number: 102263
- B. Amount of Grant: \$42,981
- C. Project Title: Assessment of Genetic Diversity and Connectivity in Fish Replenishment Areas in the Hawaiian Yellow Tang (*Zebrasoma flavescens*).
- D. Guarantee:
- E. Award Period: From 2/23/04 To 12/31/04
- F. Period Covered by this Report: From 2/23/04 To 6/1/04
- G. Summary of Progress and Expenditures to Date:

1. Work Accomplishments: (as related to project objectives and schedule for completion)

a. In accordance with the goals and objectives outlined in the revised proposal, we have completed;

1. Construction of a microsatellite DNA library for the Yellow tang. This library consists of four bacterial clone libraries, each enriched for a different tetranucleotide microsatellite motif. The library is housed at Washington State University in a -80°C freezer. Construction of the library also entailed the design and optimisation of polymerase chain (PCR) primers for approximately 35 polymorphic microsatellite sequences, which were identified during the library construction process.
2. Sample collection and DNA extraction from approximately 400 Yellow tangs collected from Fish Replenishment Areas along the west coast of Hawai'i. Several samples were collected from the Maui Ocean Center, and DNA extracted from these also. An additional 120 adult samples were collected, and DNA extracted, representing the main juvenile collection sites.
3. Purification and quantitation of all DNA samples. The DNA has been transferred into 96 well microplate format and is stored at -20°C until required for analysis.
4. Fluorescent PCR primers have been developed and are optimised for analysis of microsatellite loci in the Yellow tang. Additionally to our proposal, we successfully tested the Yellow tang PCR primers in other coral reef species. This finding is of great interest to the Hawaiian scientific community and should form the basis of a subsequent funding proposal.
5. Microsatellite DNA electrophoresis and generation of raw data for 400 juvenile and 120 adult Yellow tang samples is complete. The final stage of this project will focus on population analysis of the microsatellite data and is expected to be completed by mid December.

b. This project is presently on track. However, we have surpassed original project objectives. In addition to analysis of all juvenile and adult samples as originally planned we have doubled sample numbers and included other species in this analysis. Continuing work includes the following;

1. Computer-aided population statistical analysis is scheduled to occupy the period between November and mid December.
2. Final report writing and manuscript preparation is scheduled in December.

## 2. Applications:

a. Presentations have been prepared and submitted to all of the quarterly HCRI meetings to date and the final project report is in preparation. Noakes travelled from Washington State University Vancouver to Honolulu in order to present the previous presentation. A short information session was also presented to a college class by Noakes during this period. A manuscript is in preparation for publication submission, explaining the characterisation of the microsatellite library and detailing variability at microsatellite loci in the Yellow tang. A short communication is also in preparation, detailing the usefulness of Yellow Tang microsatellite PCR primers in other coral reef fish species. Both of these will be submitted for publication at the completion of the project in December.

b. Interest generated during Noakes' visit to Hawai'i is expected to foster future collaborative links between WSU Vancouver and UH, and also between James Cook University, Australia. The additional research findings in other coral reef species will benefit the Hawaiian research community and some preliminary research is presently being undertaken in Hawai'i that is based on the research findings of our project thus far. We anticipate a subsequent funding proposal to continue this aspect of the present project and also, to develop collaborative links in this area. Research and findings of the present project have been referenced in an NSF grant proposal being submitted by colleagues.

c. The following data has been generated but not yet published;

1. DNA sequence of approximately 100 microsatellite clones from the library.
2. Approximately 270 oligonucleotide PCR primer sequences have been developed for Yellow tang microsatellites. Of these, 34 have been synthesised for use in fluorescent PCR analysis.
3. Eight Yellow tang individuals have been tested for each of the 34 synthesised primers and examined for polymorphism (see attached example agarose gel electrophoresis).
4. Nine of the above PCR primers have been selected and are presently being optimised and successfully used for fluorescent microsatellite PCR analysis of 400 juvenile and 120 adult Yellow tang samples.
5. Three PowerPoint presentations have been prepared, presented, and copies submitted to HCRI. This research has also contributed to public brochures developed and distributed by HCRI.

## 3. Expenditures:

a. Expenditures scheduled for this period include;

1. Consumables and reagents for Yellow tang tissue collection and DNA extraction.
2. Consumables, reagents and fluorescent primers for PCR.
3. Consumables and reagents for ABI3100 automated genetic analyser.
4. Desktop computer for statistical population analysis and literature preparation.

b. Actual expenditures this period include all of the above. Project budget is on schedule.

Prepared By: \_\_\_\_\_ - 6/10/2004  
Signature of Principal Investigator Date

## NOTICE

Subsequently, all NOAA COP recipients with approved grants will be asked to file a COP Annual Progress Report in the specified format. The first section of the proposed format is taken from the COP implementation plan and has some advantages in that previously-funded investigators will be familiar with the format. Consistency in reporting requirements for competitive research grant programs is desirable and this is behind COP's efforts in proposing a standardized format. This annual report format will enable COP program staff to monitor each project supported by an award.

Public reporting burden for this collection of information is estimated to average 300 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed and completing and reviewing the collection of information.

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